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Streptomyces Isolates from the Soil of an Ancient Irish Cure Site, Capable of Inhibiting Multi-Resistant Bacteria and Yeasts

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Abstract: Traditional Irish medicines are often intertwined with ritual and spirituality, making it difficult to substantiate the validity of their claims. In this manuscript, we use molecular and microscopic techniques to investigate some microorganisms that might be responsible for the reputed healing properties of an ancient Irish soil cure known as the Blessed clay from a site in Boho in the West Fermanagh Scarplands. We previously reported the isolation of an antibiotic producing bacteria from this soil. In this report, we characterize the antibiotic activity of a further six isolates of *Streptomyces* from this source. Two of these isolates inhibit the growth of multi-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*, two inhibit the growth of the yeast *Starmerella bombicola*, and two have as yet undetermined activity. Genetic analysis of these *Streptomyces* reveals the potential to synthesize varieties of antibiotics similar to cypemycin, griseochelin, macrolactams, and candicidin. From these observations, we suggest that part of the medicinal reputation of the Blessed clay may lie in the diversity of antimicrobial producing *Streptomyces* isolated from this soil. These findings highlight the potential for antibiotic discovery in this area.

Keywords: ethnopharmacology; soil; antimicrobial; *streptomyces*; antibiotics; MRSA; traditional medicine

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1. Introduction

The record of traditional Irish folk medicine, essentially passed on by word of mouth, is fading from the living landscape. The principal reasons for this are the advancing age of the population, the sharp decline in the use of traditional medicine, and of course, the reliability of modern medicines. However, in a reversal of fortune, researchers are now looking towards traditional medicines as a potential source of new pharmaceutical compounds [1–5].This necessity is partly due to a reduction in the supply of new antibiotics caused by years of under-investment [6,7]. Indeed, the World Health Organization (WHO), in their Global Pathogen Priority (GPP) catalogue, identified a group of multi-resistant pathogens, including methicillin resistant *Staphylococcus aureus* and multi resistant *Pseudomonas aeruginosa*, as priority targets for the research and development of new antibiotics [8]. These challenges have prompted researchers to pursue more innovative methods

of drug discovery. One of these approaches, pioneered by Geoffrey Cordell, was the analysis of traditional folk-medicine. Geoffrey discovered that traditional medicines yielded far more successful lead compounds in the development of anticancer drugs than random plant screenings [9]. These ideas were quickly adopted by other researchers. Pioneering work by the group of Julian Davies in British Columbia discovered that the sacred clay, used for millennia by the Heiltsuk peoples of Kisameet Bay, was able to combat many multi-resistant hospital pathogens [3]. In another part of the globe, researchers in Jordan attributed the therapeutic activity of 'Red clay' to a high proportion of antibiotic producing bacteria found in the soil [10]. Researchers have discovered that antibiotic producing organisms could also have a close relationship with plants. This was the case for *Streptomyces* sp. strain Y3111, which lives in association with the traditional Chinese medicinal plant *Heracleum souliei* and produces anti-tubercular (anti-BCG) compounds named heraclemycins [1]. Some researchers have even reconstituted ancient medicines from old texts that have been proven to be effective against antibiotic resistant organisms such as methicillin resistant *Staphylococcus aureus* (MRSA) [2]. The common theme running through many of these discoveries has been the presence of *Streptomyces*. Together with other closely related genera, these organisms produce as much as 50% of the world's current antibiotics, as well as anticancer, antiparasitic, and antifungal compounds [11].

The West Fermanagh Scarplands are a remote range of limestone hills on the border between N. Ireland and Eire. This area has a long tradition of folk cures associated with rocks, soils, and wells, some of which are named after the diseases they cure, such as jaundice, scurvy, and ague. One of the traditional medicines from this area concerns the soil that covers the grave of a cleric known as Fr McGirr. Fr McGirr belonged to a group of priests who emerged at the end of the penal laws in Ireland. Many of these people were familiar with both spirituality and local folk healing practices [12]. In his later years' Fr McGirr stated that the soil that covered him would be able to cure the same diseases as it did when he was alive. This soil or 'Blessed clay', as it is described by locals, is wrapped in cloth and is given for ailments as diverse as toothache or tuberculosis. In our previous research, we isolated one species of *Streptomyces* from this soil that inhibited several antibiotic resistant pathogens [13]. Our current investigation involves the isolation and characterization of other *Streptomycetes* isolated from this soil. We also used the antibiotic prediction software antiSMASH [14,15], in combination with in vitro antimicrobial analysis, to optimize the potential antimicrobial identification from the genomes of these isolates.

2. Materials and Methods

2.1. Microorganism Strains

Methicillin resistant *Staphylococcus aureus* (MRSA) (ATCC 43300), *Pseudomonas aeruginosa* (PA01), and *Starmerella bombicola* were kindly provided by Prof. I.M. Banat (Ulster University). *Streptomyces* sp. myrophorea isolate McG1 (NCTC 14177, DSM 107716) was previously isolated from a soil sample in the Boho region of the West Fermanagh Scarplands and is also referred to as *Streptomyces* strain McG1 in this manuscript [13].

Streptomyces isolates McG2 up to McG7 were also isolated from the same soil sample, as described above. One gram of this soil sample was diluted in 1 mL sterile water, vortexed, and cultured on International Streptomyces Project (ISP) 2 agar (1/5th strength) and starch agar (Oxoid, Hampshire, UK), as previously described [13]. *Streptomyces* isolates were characterized by observing their distinctive colony morphology (small, embedded colonies with powdery surfaces) on ISP2 media. These species were further identified using whole genome sequencing. Stocks of these *Streptomyces* were frozen at –80 °C in 18% glycerol after their initial isolation.

2.2. Antimicrobial Tests

Antimicrobial tests followed a modified agar overlay method [16]. Briefly, an agar plug/core of *Streptomyces* that had grown on soy flour mannitol (SFM) agar for 9–15 days was placed within wells made in 15 mL of 1.5% agar. This was overlaid with Muller–Hinton

agar containing pre-diluted test organism at 42 °C in the case of *P. aeruginosa*, tryptic soy agar (TSB) in the case of *S. aureus*, and Sabouraud dextrose agar in the case of *S. bombicola*. In cases where the overlay methods were not consistent, a sterile swab was used to apply a dilution of 1×10^6 cfu/mL of test organism. Zones of inhibition in the confluent growth of test pathogens was deemed to indicate antimicrobial activity. Inhibition zones less than 1mm from agar core were recorded as resistant.

Antibiograms followed the Kirby–Bauer protocol [17]. Organisms were grown on Muller–Hinton agar and tested against a variety of antibiotic discs, which were placed on top of the agar. Agar plates were incubated overnight at 37 °C. The presence of a zone of inhibition greater than 1 mm from the edge of the disk indicated inhibition.

2.3. Molecular Biology

2.3.1. Genome Sequencing

Genome sequencing of *Streptomyces* was performed by MicrobesNG (Birmingham, UK). The DNA was extracted from *Streptomyces* using a Qiagen DNA extraction Kit 200 (Qiagen, Venlo, The Netherlands) with modifications. A pure culture of *Streptomyces* grown on an agar plate was suspended in 500 mL PBS in lysing matrix in a 2 mL tube (MP biomedical, Santa Ana, CA, USA). Bacterial cells were lysed by fast prep (60 for 40 s (×2). The suspension was centrifuged, and the supernatant was transferred to 2 mL Eppendorf tubes. Next, 500 µL of ATL lysis buffer and 80 µL of proteinase were added and incubated for 10 min at 70 °C. A further 16 µL RNase and 500 µL were then added to the buffer and incubated at 70 °C for 7 min. A further 600 µL of ethanol was then added. The suspension was transferred to the mini spin column and centrifuged at 6010× *g* for 1 min. This step was repeated until the whole suspension had passed through. The column was washed twice, and the DNA was eluted in 50 µL of elution buffer. Extracted DNA was quantified with a Nano Drop spectrophotometer.

Genomic DNA libraries were prepared using Nextera XT Library Prep Kit (Illumina, San Diego, CA, USA). The manufacturers protocol was changed slightly by adding two nanograms of DNA instead of one as an input, and the PCR elongation time was changed from 30 s to one minute. DNA quantification and library preparation were performed on a Hamilton Microlab STAR automated liquid handling system. Pooled libraries were quantified using the Kapa Biosystems Library Quantification Kit for Illumina on a Roche light cycler 96 qPCR machine. Libraries were sequenced on the Illumina HiSeq using a 250 bp paired end protocol. Reads were adapter trimmed using Trimmomatic 0.30 with a sliding window quality cut off of Q15 [18]. De novo assembly was performed on samples using SPAdes version 3.7 [19], and contigs were annotated using Prokka 1.11 [20]. The genomes of *Streptomyces* spp. isolates McG2, McG3, McG5, McG6, McG7, and McG8 were sequenced. The sequence of *Streptomyces* sp. isolate McG1 was already reported in another study [13].

2.3.2. Genome Deposition

Whole genome sequences of *Streptomyces* spp. isolates McG2, McG3, McG5, McG6, McG7, McG8 were deposited in DDBJ/ENA/GenBank in the Bioproject PRJNA433829 under the following accession numbers: McG2, JAAXYB000000000; McG3, JAAXYC000000000; McG5: JAAXYD000000000; McG6, JAAXYE000000000; McG7, JAAXYF000000000; and McG8, JAAXYG000000000. The versions described here are the first versions and have been assembled to the contig level.

2.3.3. Taxonomic Position of Streptomyces Isolates Using a Maximum-Likelihood Phylogeny

A multi-locus phylogeny was reconstructed by the method reported previously [13]. Briefly, whole genome sequences (WGS) for all *Streptomyces* were retrieved autonomously from GenBank. Genomes were annotated in PROKKA [20], and the resulting translated coding domain sequences were used to generate a multi-locus, concatenated alignment

of 400 proteins in PhyloPhlan [21]. Proteins used for the alignment were those shown to be conserved among bacterial genera [21]. A maximum-likelihood phylogeny was reconstructed in FastTree and rendered in iTOL [22,23]. The robustness of the phylogeny was assessed using 1000 bootstrap replications. The phylogeny was reconstructed with 921 *Streptomyces* genomes (including McG designated isolates). The phylogeny was outgroup rooted along the lineage leading to Frankia (five species). The full list of species in the phylogeny can be found in Supplementary Data (File S1). Genome-to-genome distance calculations were performed in GGDC web server (http://ggdc.dsmz.de/ server accessed on 22 May 2021) using formula 3 to account for the use of draft genomes.

2.3.4. Secondary Metabolite Analysis

Gene clusters known to be involved in secondary metabolite biosynthesis, self-immunity, or resistance were identified using Antibiotics and Secondary Metabolite Analysis Shell (anti-SMASH) version 4.0.0 [14]. The relaxed metabolite matching parameter in antiSMASH was chosen for comparison of the *Streptomyces* genomes. This detects well-defined clusters containing all required parts of a biosynthetic gene cluster, as well as partial clusters missing one or more functional parts. The GenBank sequence files (from Prokka annotation) were submitted to the web interface selecting all extra features of annotation.

3. Results

3.1. Microbial Characterization

We isolated six *Streptomyces* from the 'Blessed clay' of Fr McGirr, which we labelled as *Streptomyces* spp. McG2 to McG8 (herein referred to as McG2 to McG8). These were identified from colony morphology, microscopy, and whole genome sequencing. We have previously characterized *Streptomyces* sp. myrophorea isolate McG1 (NCTC 14177) [13].

Streptomyces spp. McG2 to McG8 grew well on ISP2, PDA, and starch agar. All isolates were characterized by small, embedded colonies with powdery surfaces that emitted earthy odors. All of these *Streptomyces* isolates had a high salt (NaCl) tolerance (Table 1).

Table 1. Characterization of *Streptomyces* isolates from Boho, County Fermanagh.

Isolate	Colonies	Spores	NaCl (*w*/*v*) Tolerance in Growth Media
Streptomyces sp. isolate McG1	pale green to green	green to pale	spores to 6%, grows up to 10%
McG2	pale green to green	green to pale	grows and spores up to 10%
McG3	pale green to green	green to pale	spores to 6%, grows up to 10%
McG5	pale green to light brown	pale green to brown	grows and spores up to 10%
McG6	pale green to light brown	pale green to brown	grows and spores up to 10%
McG7	pale green to light brown	white to brown	spores to 8%, grows up to 10%
McG8	pale green to light brown	brown	spores to 6%, grows up to 10%

Microscopically, all *Streptomyces* isolates were Gram-positive, filamentous, with branching and aerial hyphae that produced spores.

3.2. Antimicrobial Activities of the Isolated Streptomyces Strains

The antimicrobial activity of the *Streptomyces* isolates was tested against methicillin resistant *Staphylococcus aureus* (MRSA) (ATCC 43300), *Pseudomonas aeruginosa* (PA 01), and the yeast *Starmerella bombicola* on agar plates using a modified version of the Lehrer assay (Figure 1 and Table 2). The antibiotic resistance profiles of the test organisms are shown in Table 3.

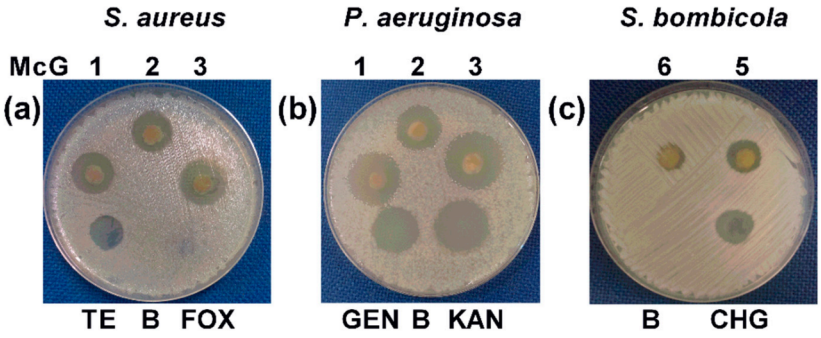

Figure 1. Boho *Streptomyces* spp. isolates McG1, McG2, and McG3 tested for inhibitory activity against (**a**) MRSA 43,300 (**b**) *P. aeruginosa* (PA01). Isolates McG5 and McG6 tested for inhibitory activity against (**c**) *S. bombicola*. Control wells contain gentamicin (GEN 20 µg), kanamycin (KAN 20 µg), teicoplanin (TE 20 µg), cefoxitin (FOX 20 µg), chlorhexidine (CHG, 40 µg), and control blank (B). Tests performed three or more times.

Table 2. The inhibitory potential of the *Streptomyces* isolates from the Boho clay against *S. aureus* (MRSA 43300), *P. aeruginosa* (PA01), and *S. bombicola*. Inhibition zones recorded in millimeters (mm), resistant (R). Tests were repeated a minimum of three times.

Isolate	*P. aeruginosa*	*S. aureus*	*S. bombicola*
Streptomyces sp. isolate McG1	18.3 ± 0.6	17.7 ± 0.6	R
McG2	16.5 ± 0.5	17.3 ± 0.6	R
McG3	19.0 ± 1.0	20.3 ± 0.6	R
McG5	R	R	14.7 ± 2.1
McG6	R	R	11.3 ± 1.5
McG7	R	R	R
McG8	R	R	R

Table 3. The antibiotic resistance profiles of the test organisms *S. aureus* (MRSA 43300), *P. aeruginosa* (PA 01), and *S. bombicola*. Zones of inhibition less than 1 mm from antibiotic disc (Oxoid) were scored as resistant. Antibiotic doses in µg. Resistant (R), not applicable (NA).

Antibiotic (µg)	*S. aureus*	*P. aeruginosa*	*S. bombicola*
Teicoplanin 20	13.7 ± 0.6	NA	NA
Cefoxitin 20	R	NA	NA
Gentamicin 20	NA	19.3 ± 0.6	NA
Kanamycin 20	NA	21.7 ± 0.6	NA
Chlorhexidine 40	NA	NA	15.0 ± 1.0
Vancomycin 5	17.0 ± 1.0	R	NA
Ampicillin 10	R	R	NA
Ciprofloxacin 1	20 0 ± 1.0	23.0 ± 1.0	NA
Tetracycline 30	33 0 ± 3.0	R	NA

3.3. Genome Assembly of Streptomyces Isolates from the Boho Clay

Streptomyces genomes were assembled de-novo by MicrobesNG (Birmingham University, Birmingham, UK). The k-mer spectrums of contigs larger than 2000 bp were calculated based on an approach described previously [24]. For four of the isolates, sets of low coverage contigs with k-mer spectrums significantly different from the five largest contigs in the assembly were identified as probable contaminants. All small contigs of less than 500 bp were removed from the assemblies (Table 4).

Table 4. Summary statistics of the genomes of *Streptomyces* isolates. Number of biosynthetic gene clusters is based on antiSMASH analysis.

Isolate	Size (Mb)	Contig Numbers	Contig N50 (Kb)	Longest Contig (Kb)	Biosynthetic Gene Clusters
McG2	6.912	1189	9.0	70.7	25
McG3	8.720	586	23.6	125.6	39
McG5	7.026	1079	11.8	70.7	27
McG6	6.874	1524	7.3	62.2	24
McG7	7.143	465	26.0	82.9	12
McG8	7.304	653	18.3	62.7	12

3.4. Prediction of Antibiotic Gene Cluster Similarities

The potential of the *Streptomyces* isolates to produce antibiotics and other secondary metabolites was predicted using antiSMASH (version 4), which compared our unknown gene sequences with previously documented antibiotic/secondary metabolite biosynthetic gene clusters (BGC). These BGCs contain the genes necessary to produce secondary metabolites, including enzymes and pathway-specific regulatory genes.

We identified many BCGs in the genomes of the six strains of *Streptomyces* from the Blessed clay using antiSMASH. Many of the gene cluster similarities identified are common in *Streptomyces*, including albaflavenone (a sesquiterpene antibiotic) [25], alkylresorcinol [26], desferrioxamine (a siderophore that mitigates cytotoxicity to doxorubicin) [27], ectoine [28], isorenieratene (a bacterial chlorophyll) [29], and melanin [30]. These compounds are recorded here because of their potential synergy with antibiotics [31]. Most importantly, there were also similarities between the gene sequences we identified in these *Streptomyces* and several antibiotic gene synthesis clusters, including those for polycyclic tetramate macrolactams (SGR PTM), candicidin, griseochelin, cypemycin, and cyslabdan [31–35] (Table 5).

Table 5. Biosynthetic gene cluster (BGC) similarities between *Streptomyces* isolates and known antimicrobial synthesis genes. The biosynthetic gene clusters are hyperlinked to their corresponding matches in the Minimum Information about a Biosynthetic Gene cluster (MIBiG) repository. Abbreviation: *Streptomyces griseus* polycyclic tetramate macrolactam (SGR PTM). * *Streptomyces* sp. McG1 [14].

Gene Cluster	Shared	BGC Data Link	*Streptomyces* Isolates McG						
			1 *	2	3	5	6	7	8
Cypemycin	77%	BGC0000582_c1	✔		✔	✔			
Candicidin A	66%	BGC0000034_c1		✔		✔	✔		
Griseochelin	84%	BGC0001821_c1	✔		✔				
SGR_PTMs	83%	BGC0001043_c1	✔		✔				
Cyslabdan	81%	BGC0001910_c1						✔	✔

AntiSMASH identified several gene clusters in whole genome sequences of *Streptomyces* isolates McG1 and McG5 with similarities to the lantibiotic cypemycin (77%) (Figure 2a).

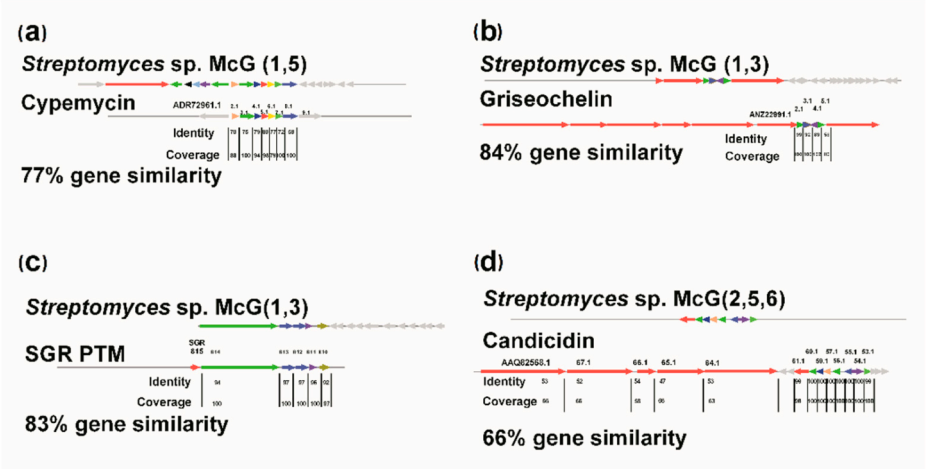

Figure 2. Biosynthetic cluster similarities between *Streptomyces* McG isolates and known antibiotic genes for (**a**) cypemycin, (**b**) griseochelin, (**c**) SGR PTM, and (**d**) candicidin.

Genes from *Streptomyces* sp. McG3 showed similarities to the griseochelin (zincophorin) biosynthesis cluster (Figure 2b). Boho *Streptomyces* sp. isolates McG1 and McG3 had similar gene sequences to biosynthesis clusters for SGR PTM, a polytetramic macrolactam [36] (Figure 2c). *Streptomyces* strains McG2, 5, and 6 had gene similarities to biosynthesis clusters for candicidin, an antifungal antibiotic [37] (Figure 2d). Finally, the WGS of *Streptomyces* isolates McG7 and McG8 showed similarities to gene clusters for cyslabdan [32].

3.5. Streptomyces Isolate Phylogeny

The relationships between the *Streptomyces* isolates from the Boho clay were calculated using the maximum-likelihood of relatedness. This placed isolates McG2, McG5, and McG6 alongside the *S. albidoflavus* clade, suggesting that these were likely variants (Figure 3a). Indeed, GGDC calculations supported this, showing that isolates McG2, McG5, and McG6 were closely related to other *S. albidoflavus* strains with DDH scores of 90, 94, and 90, respectively. Moreover, probability scores of being the same species (i.e., *S. albidoflavus*) being >99% in all cases. Phylogenetically, *Streptomyces* isolate McG3 (Figure 3b) sits alongside *Streptomyces* sp. CNB-091, a marine *Streptomyces* [38].

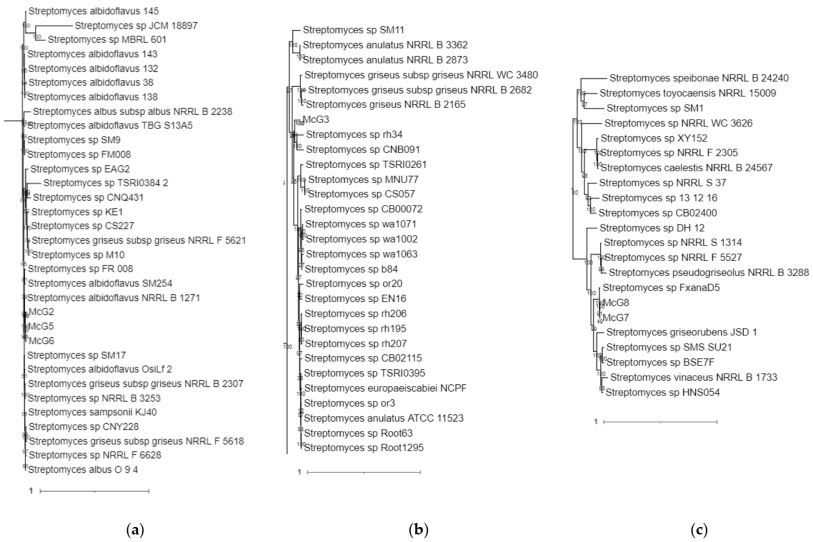

Figure 3. Phylogenetic relationships between (**a**) *Streptomyces* isolates McG2, McG5, and McG6; (**b**) *Streptomyces* isolate McG3; and (**c**) *Streptomyces* isolates McG7 and McG8 using maximum-likelihood phylogeny. Phylogenies were reconstructed in FastTree MP and rendered in iTOL.

The closest species to *Streptomyces* sp. McG7 and McG8 was *Streptomyces* sp. FxanaD5 (Figure 3c). These new isolates were added to the data set of [5,13]. The full phylogeny is available from the authors on request.

4. Discussion

There has been an increasing interest in the potential of traditional medicines to provide new medicines over the past few decades [3]. However, many of the older tradition medicines are hard to decipher because of their complicated relationship with rituals and mythology. Using a series of microbiological methods, we isolated a group of *Streptomyces* from the Blessed clay of Fr McGirr in the West Fermanagh Scarplands. Several of these isolates inhibited the growth of multi-resistant pathogens and yeasts. These add to the growing list of *Streptomyces* sp. isolated from traditional medicines that are able to produce antibiotics against fungi and bacteria [39]. We were also able to predict the potential synthesis of a variety of antimicrobial substances from these isolates. We think that these discoveries may provide a possible explanation as to why this soil has historically been used as a folk medicine. There is also the possibility that other types of bacteria could also be associated with the reputed antimicrobial activity of this soil; however, in this study we concentrated solely on *Streptomyces*. Additionally, the activity of the Blessed clay could equally be based on some type of clay mineral, such as Montmorillonite [40,41] or sulfur- and iron-reducing bacteria [42]. However, the Blessed clay in this study has a silty-loam consistency rather than clay-like, similar to the rest of the soil found in this area.

Given the continuous spiritual significance of this site, we assume many of the folk medicines found in this area are derived from much older cultures. Since this particular traditional soil medicine was passed down by word of mouth, we are still uncertain of the conditions under which it could yield its full therapeutic potential. Therefore, even though we managed to induce the production of antibiotics from *Streptomyces* isolated from this soil under laboratory conditions, this might still not be the complete repertoire of its activity.

We are aware that limestone areas similar to Boho have long been regarded as a rich source of antibiotic producing *Streptomyces* [43–45]. The *Streptomyces* species identified from the Blessed clay sample is grouped into three main clusters. *Streptomyces* spp. McG7 and McG8 were found to be closely related to both *Streptomyces* sp. FxanaD5 and *S. griseorubens* JSD-1. *Streptomyces* sp. isolate McG3 was found to be closely related to *Streptomyces* sp. CNB-091, a marine *Streptomyces* 38]; *S. griseus subsp griseus* NRRL B 2682; and *Streptomyces* sp. McG1 (*Streptomyces* sp. myrophorea) [13]. In the last grouping, *Streptomyces* spp. isolates McG2, McG5, and McG6 were closely related to *S. albidoflavus*. In many cases, these Boho *Streptomyces* isolates were closely related to marine species.

In terms of antibiotic potential of the Boho clay isolates, antiSMASH software predicted that *Streptomyces* isolates McG2, McG5, and McG6 had biosynthetic gene clusters similar to those for candicidin, an antifungal compound [37]. It has been reported that the majority of isolates exclusively producing candicidin come mainly from which matches with the identity of *Streptomyces* sp. isolates McG2, McG5, and McG6 [46]. However, our in vitro inhibition tests showed that only *Streptomyces* isolates McG5 and McG6 expressed antifungal activities, whilst McG2 did not show any activity.

In vitro testing showed that only *Streptomyces* isolates McG1, McG2, and McG3 had any inhibitory effect on multi-resistant bacteria. The identities of the compounds responsible for this activity were difficult to discern from the antiSMASH predictions since there were several biosynthetic gene clusters identified for each isolate. *Streptomyces* isolates McG1 and McG3 encoded antibiotic synthesis clusters similar to *Streptomyces griseus* polytetramic macrolactam (SGR PTM), an antifungal and antioxidant [36]. *Streptomyces* isolates McG1 and McG3 also encoded gene synthesis clusters similar to those of griseochelin (zincophorin), a carboxylic acid antibiotic that is active against Gram-positive bacteria and viruses. *Streptomyces* isolates McG1 and McG5 also have gene synthesis clusters for cypemycin, a Gram-positive antibiotic that also has activity against leukemia [34]. Finally,

a gene cluster from *Streptomyces* isolates McG7 and McG8 showed similarity to those previously identified for the synthesis of cyslabdan, an antimicrobial potentiator [33]. Several researchers have also shown that some species of *Streptomyces* that only produce low levels of antibiotic when cultivated alone are able to induce other species to express new antibiotics [47]. This may be an interesting direction to follow in future research.

Altogether, the *Streptomyces* species isolated here are effective against multi-resistant bacteria and fungi. It may be possible that this spectrum might extend to anticancer and antiviral activity, but this is still the subject of ongoing research. This new source of *Streptomyces* strains may be a useful resource in helping to replenishing the ever-decreasing range of antibiotics capable of combating multi-resistant microbial pathogens.

5. Conclusions

We identified a group of *Streptomyces* from the Blessed clay in the Boho region of West Fermanagh. We have shown through genome analysis and by in vitro tests that some of these *Streptomyces* have the ability to produce antimicrobial compounds that inhibit the growth of clinically significant multi-resistant pathogens in vitro. We do not know how to activate this soil in situ, but we feel that the abundance of *Streptomyces* discovered in the Boho clay might be responsible for its reputed healing abilities. We think that these findings might provide a basis for future antibiotic discovery.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11114923/s1, File S1: List of species used in taxonomic positioning of *Streptomyces* isolates using a maximum-likelihood phylogeny analysis.

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References

1. Liu, M.; Abdel-Mageed, W.M.; Ren, B.; He, W.; Huang, P.; Li, X.; Bolla, K.; Guo, H.; Chen, C.; Song, F.; et al. Endophytic Streptomyces Sp. Y3111 from Traditional Chinese Medicine Produced Antitubercular Pluramycins. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 1077–1085. [CrossRef] [PubMed]
2. Harrison, F.; Roberts, A.E.L.; Gabrilska, R.; Rumbaugh, K.P.; Lee, C.; Diggle, S.P. A 1000-Year-Old Antimicrobial Remedy with Antistaphylococcal Activity. *mBio* **2015**, *6*. [CrossRef] [PubMed]
3. Behroozian, S.; Svensson, S.L.; Davies, J. Kisameet Clay Exhibits Potent Antibacterial Activity against the ESKAPE Pathogens. *mBio* **2016**, *7*. [CrossRef] [PubMed]
4. Wagner, C.S.; De Gezelle, J.; Robertson, M.; Robertson, K.; Wilson, M.; Komarnytsky, S. Antibacterial Activity of Medicinal Plants from The Physicians of Myddvai, a 14th Century Welsh Medical Manuscript. *J. Ethnopharmacol.* **2017**, *203*, 171–181. [CrossRef]
5. Quinn, G.A.; Abdelhameed, A.M.; Alharbi, N.K.; Cobice, D.; Adu, S.A.; Swain, M.T.; Castro, H.C.; Facey, P.D.; Bakshi, H.A.; Tambuwala, M.M.; et al. The Isolation of a Novel Streptomyces Sp. CJ13 from a Traditional Irish Folk Medicine Alkaline Grassland Soil That Inhibits Multiresistant Pathogens and Yeasts. *Appl. Sci.* **2021**, *11*, 173. [CrossRef]
6. Kapoor, G.; Saigal, S.; Elongavan, A. Action and Resistance Mechanisms of Antibiotics: A Guide for Clinicians. *J. Anaesthesiol. Clin. Pharmacol.* **2017**, *33*, 300–305. [CrossRef]
7. Ribeiro da Cunha, B.; Fonseca, L.P.; Calado, C.R.C. Antibiotic Discovery: Where Have We Come from, Where Do We Go? *Antibiotics* **2019**, *8*, 45. [CrossRef]
8. Tacconelli, E.; Carrara, E.; Savoldi, A.; Harbarth, S.; Mendelson, M.; Monnet, D.L.; Pulcini, C.; Kahlmeter, G.; Kluytmans, J.; Carmeli, Y.; et al. Discovery, Research, and Development of New Antibiotics: The WHO Priority List of Antibiotic-Resistant Bacteria and Tuberculosis. *Lancet Infect. Dis.* **2018**, *18*, 318–327. [CrossRef]
9. Cordell, G.A.; Farnsworth, N.R.; Beecher, C.W.W.; Doel Soejarto, D.; Kinghorn, A.D.; Pezzuto, J.M.; Wall, M.E.; Wani, M.C.; Cobb, R.R.; O'Neill, M.J.; et al. Novel Strategies for the Discovery of Plant-Derived Anticancer Agents. In *Anticancer Drug Discovery and Development: Natural Products and New Molecular Models. Developments in Oncology*; Valeriote, F.A., Baker, L.H., Eds.; Springer: Boston, MA, USA, 1994; ISBN 978-1-4613-6118-3.
10. Falkinham, J.O., 3rd; Wall, T.E.; Tanner, J.R.; Tawaha, K.; Alali, F.Q.; Li, C.; Oberlies, N.H. Proliferation of Antibiotic-Producing Bacteria and Concomitant Antibiotic Production as the Basis for the Antibiotic Activity of Jordan's Red Soils. *Appl. Environ. Microbiol.* **2009**, *75*, 2735–2741. [CrossRef]
11. Antoraz, S.; Santamaría, R.I.; Díaz, M.; Sanz, D.; Rodríguez, H. Toward a New Focus in Antibiotic and Drug Discovery from the Streptomyces Arsenal. *Front. Microbiol.* **2015**, *6*, 461. [CrossRef]
12. Donnelly, C.J. Masshouses and Meetinghouses: The Archaeology of the Penal Laws in Early Modern Ireland. *Int. J. Hist. Archaeol.* **2004**, *8*, 119–132. [CrossRef]
13. Terra, L.; Dyson, P.J.; Hitchings, M.D.; Thomas, L.; Abdelhameed, A.; Banat, I.M.; Gazze, S.A.; Vujaklija, D.; Facey, P.D.; Francis, L.W.; et al. A Novel Alkaliphilic Streptomyces Inhibits ESKAPE Pathogens. *Front. Microbiol.* **2018**, *9*, 2458. [CrossRef] [PubMed]
14. Medema, M.H.; Blin, K.; Cimermancic, P.; de Jager, V.; Zakrzewski, P.; Fischbach, M.A.; Weber, T.; Takano, E.; Breitling, R. AntiSMASH: Rapid Identification, Annotation and Analysis of Secondary Metabolite Biosynthesis Gene Clusters in Bacterial and Fungal Genome Sequences. *Nucleic Acids Res.* **2011**, *39*, W339–W346. [CrossRef] [PubMed]
15. Adamek, M.; Spohn, M.; Stegmann, E.; Ziemert, N. Mining Bacterial Genomes for Secondary Metabolite Gene Clusters. *Methods Mol. Biol.* **2017**, *1520*, 23–47. [CrossRef]
16. Lehrer, R.I.; Rosenman, M.; Harwig, S.S.; Jackson, R.; Eisenhauer, P. Ultrasensitive Assays for Endogenous Antimicrobial Polypeptides. *J. Immunol. Methods* **1991**, *137*, 167–173. [CrossRef]
17. Bauer, A.W.; Kirby, W.M.; Sherris, J.C.; Turck, M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *Am. J. Clin. Pathol.* **1966**, *45*, 493–496. [CrossRef]
18. Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **2014**, *30*, 2114–2120. [CrossRef]
19. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A.A.; Dvorkin, M.; Kulikov, A.S.; Lesin, V.M.; Nikolenko, S.I.; Pham, S.; Prjibelski, A.D.; et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* **2012**, *19*, 455–477. [CrossRef]
20. Seemann, T. Prokka: Rapid Prokaryotic Genome Annotation. *Bioinformatics* **2014**, *30*, 2068–2069. [CrossRef]
21. Segata, N.; Börnigen, D.; Morgan, X.C.; Huttenhower, C. PhyloPhlAn Is a New Method for Improved Phylogenetic and Taxonomic Placement of Microbes. *Nat. Commun.* **2013**, *4*, 2304. [CrossRef]
22. Price, M.N.; Dehal, P.S.; Arkin, A.P. FastTree 2–Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE* **2010**, *5*. [CrossRef]
23. Letunic, I.; Bork, P. Interactive Tree of Life (ITOL) v3: An Online Tool for the Display and Annotation of Phylogenetic and Other Trees. *Nucleic Acids Res.* **2016**, *44*, W242–W245. [CrossRef]
24. Swain, M.T. Fast Comparison of Microbial Genomes Using the Chaos Games Representation for Metagenomic Applications. *Procedia Comput. Sci.* **2013**, *18*, 1372–1381. [CrossRef]
25. Gurtler, H.; Pedersen, R.; Anthoni, U.; Christophersen, C.; Nielsen, P.H.; Wellington, E.M.; Pedersen, C.; Bock, K. Albaflavenone, a Sesquiterpene Ketone with a Zizaene Skeleton Produced by a Streptomycete with a New Rope Morphology. *J. Antibiot.* **1994**, *47*, 434–439. [CrossRef]

26. Kanda, N.; Ishizaki, N.; Inoue, N.; Oshima, M.; Handa, A. DB-2073, a New Alkylresorcinol Antibiotic. I. Taxonomy, Isolation and Characterization. *J. Antibiot.* **1975**, *28*, 935–942. [CrossRef]
27. Challis, G.L.; Hopwood, D.A. Synergy and Contingency as Driving Forces for the Evolution of Multiple Secondary Metabolite Production by Streptomyces Species. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 14555. [CrossRef]
28. Galinski, E.A.; Pfeiffer, H.P.; Truper, H.G. 1,4,5,6-Tetrahydro-2-Methyl-4-Pyrimidinecarboxylic Acid. A Novel Cyclic Amino Acid from Halophilic Phototrophic Bacteria of the Genus Ectothiorhodospira. *Eur. J. Biochem.* **1985**, *149*, 135–139. [CrossRef]
29. Krugel, H.; Krubasik, P.; Weber, K.; Saluz, H.P.; Sandmann, G. Functional Analysis of Genes from Streptomyces Griseus Involved in the Synthesis of Isorenieratene, a Carotenoid with Aromatic End Groups, Revealed a Novel Type of Carotenoid Desaturase. *Biochim. Biophys. Acta* **1999**, *1439*, 57–64. [CrossRef]
30. El-Naggar, N.E.-A.; El-Ewasy, S.M. Bioproduction, Characterization, Anticancer and Antioxidant Activities of Extracellular Melanin Pigment Produced by Newly Isolated Microbial Cell Factories Streptomyces Glaucescens NEAE-H. *Sci. Rep.* **2017**, *7*, 42129. [CrossRef]
31. Gokarn, K.; Pal, R.B. Activity of Siderophores against Drug-Resistant Gram-Positive and Gram-Negative Bacteria. *Infect. Drug Resist.* **2018**, *11*, 61–75. [CrossRef]
32. Koyama, N.; Tokura, Y.; Münch, D.; Sahl, H.-G.; Schneider, T.; Shibagaki, Y.; Ikeda, H.; Tomoda, H. The Nonantibiotic Small Molecule Cyslabdan Enhances the Potency of β-Lactams against MRSA by Inhibiting Pentaglycine Interpeptide Bridge Synthesis. *PLoS ONE* **2012**, *7*. [CrossRef]
33. Fukumoto, A.; Kim, Y.-P.; Matsumoto, A.; Takahashi, Y.; Shiomi, K.; Tomoda, H.; Ōmura, S. Cyslabdan, a New Potentiator of Imipenem Activity against Methicillin-Resistant Staphylococcus Aureus, Produced by Streptomyces Sp. K04-0144. *J. Antibiot.* **2008**, *61*, 1–6. [CrossRef]
34. Claesen, J.; Bibb, M. Genome Mining and Genetic Analysis of Cypemycin Biosynthesis Reveal an Unusual Class of Posttranslationally Modified Peptides. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 16297. [CrossRef]
35. Grafe, U.; Schade, W.; Roth, M.; Radics, L.; Incze, M.; Ujszaszy, K. Griseochelin, a Novel Carboxylic Acid Antibiotic from Streptomyces Griseus. *J. Antibiot.* **1984**, *37*, 836–846. [CrossRef]
36. Luo, Y.; Huang, H.; Liang, J.; Wang, M.; Lu, L.; Shao, Z.; Cobb, R.E.; Zhao, H. Activation and Characterization of a Cryptic Polycyclic Tetramate Macrolactam Biosynthetic Gene Cluster. *Nat. Commun.* **2013**, *4*, 2894. [CrossRef]
37. Jørgensen, H.; Fjaervik, E.; Hakvåg, S.; Bruheim, P.; Bredholt, H.; Klinkenberg, G.; Ellingsen, T.E.; Zotchev, S.B. Candicidin Biosynthesis Gene Cluster Is Widely Distributed among Streptomyces Spp. Isolated from the Sediments and the Neuston Layer of the Trondheim Fjord, Norway. *Appl. Environ. Microbiol.* **2009**, *75*, 3296–3303. [CrossRef]
38. Degen, D.; Feng, Y.; Zhang, Y.; Ebright, K.Y.; Ebright, Y.W.; Gigliotti, M.; Vahedian-Movahed, H.; Mandal, S.; Talaue, M.; Connell, N.; et al. Transcription Inhibition by the Depsipeptide Antibiotic Salinamide A. *eLife* **2014**, *3*. [CrossRef]
39. Quinn, G.A.; Banat, A.M.; Abdelhameed, A.M.; Banat, I.M. Streptomyces from Traditional Medicine: Sources of New Innovations in Antibiotic Discovery. *J. Med. Microbiol.* **2020**, *69*, 1040–1048. [CrossRef]
40. Moosavi, M. Bentonite Clay as a Natural Remedy: A Brief Review. *Iran. J. Public Health* **2017**, *46*, 1176–1183.
41. Srasra, E.; Bekri-Abbes, I. Bentonite Clays for Therapeutic Purposes and Biomaterial Design. *Curr. Pharm. Des.* **2020**, *26*, 642–649. [CrossRef]
42. Sharma, S.; Grewal, S.; Vakhlu, J. Phylogenetic Diversity and Metabolic Potential of Microbiome of Natural Healing Clay from Chamliyal (J&K). *Arch. Microbiol.* **2018**, *200*, 1333–1343. [CrossRef] [PubMed]
43. Yucel, S.; Yamac, M. Selection of Streptomyces Isolates from Turkish Karstic Caves against Antibiotic Resistant Microorganisms. *Pak. J. Pharm. Sci.* **2010**, *23*, 1–6. [PubMed]
44. Gosse, J.T.; Ghosh, S.; Sproule, A.; Overy, D.; Cheeptham, N.; Boddy, C.N. Whole Genome Sequencing and Metabolomic Study of Cave Streptomyces Isolates ICC1 and ICC4. *Front. Microbiol.* **2019**, *10*, 1020. [CrossRef] [PubMed]
45. Hamedi, J.; Kafshnouchi, M.; Ranjbaran, M. A Study on Actinobacterial Diversity of Hampoeil Cave and Screening of Their Biological Activities. *Saudi J. Biol. Sci.* **2019**, *26*, 1587–1595. [CrossRef]
46. Lewis, R.A.; Devi, J.; Green, K.; Li, J.; Hopkins, A.; Hayles, J.; Nurse, P.; Errington, J.; Allenby, N.E.E. Screening and Purification of Natural Products from Actinomycetes That Induce a "Rounded" Morphological Phenotype in Fission Yeast. *Nat. Prod. Bioprospect.* **2021**. [CrossRef]
47. Pishchany, G.; Mevers, E.; Ndousse-Fetter, S.; Horvath, D.J.; Paludo, C.R.; Silva-Junior, E.A.; Koren, S.; Skaar, E.P.; Clardy, J.; Kolter, R. Amycomicin Is a Potent and Specific Antibiotic Discovered with a Targeted Interaction Screen. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 10124. [CrossRef]